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Altered expression of fibrogenic growth factors in IgA nephropathy and focal and segmental glomerulosclerosis

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Altered expression of fibrogenic growth factors in IgA nephropathy and focal and segmental glomerulosclerosis. The profile of fibrogenic growth factor expression was assessed in biopsies from 27 patients with IgA nephropathy (IgAN), 14 focal and segmental glomerulosclerosis (FSGS) patients and 8 controls, by immunohistochemistry. Increased platelet-derived growth factor (PDGF)-A and PDGF-B expression was detected in glomeruli and in vascular structures and collapsed tubules in the interstitium. Computer assisted image analysis demonstrated increased glomerular PDGF-A in IgAN ($P < 0.05$), but not FSGS patients, compared to controls, suggesting an association with mesangial proliferation. PDGF receptors were prominent in areas of mesangial expansion and intertubular fibrosis. Significant increases in interstitial PDGF Receptor β (PDGFR-β) were detected for both IgAN ($P < 0.01$) and FSGS ($P < 0.05$) patients. Interstitial PDGFR-β expression was significantly correlated to monocyte/macrophage infiltrate ($P < 0.0001$). Increased basic fibroblast growth factor (bFGF) expression was observed segmentally in glomeruli, and in areas of tubulointerstitial damage. Higher proportions of patients with FSGS than IgAN had elevated interstitial bFGF ($P < 0.005$) and PDGF, reflecting the more severe degree of vascular and tubulointerstitial injury in FSGS patients. This study demonstrates distinct patterns of fibrogenic growth factors in IgAN and FSGS, strongly associated with the severity and type of injury.

Glomerulonephritis remains the most common cause of end-stage renal failure, and the two most common types of GN are mesangioproliferative IgA nephropathy (IgAN), and focal and segmental glomerulosclerosis and hyalinosis (FSGS). These forms of GN contrast not only in pathogenesis but also in glomerular pathology and rate of decline in renal function: IgAN is characteristically a proliferative form of GN in which only 10 to 20% of patients develop end-stage renal failure (ESRF) over a period of 5 to 20 years, while FSGS is a nonproliferative GN in which most patients develop ESRF over a period of 5 to 10 years [1]. These progressive forms of GN are characterized histologically by mesangial expansion, varying degrees of glomerular hypercellularity and glomerulosclerosis and are associated with an interstitial inflammatory cell infiltrate, fibrosis, tubular atrophy and vascular sclerosis [2].

There is increasing evidence that fibrogenic growth factors are involved in the development of glomerular and interstitial renal injury in progressive forms of GN [3–10]. These growth factors, and in particular platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and transforming growth factor β (TGFβ), are produced by multiple cells including activated macrophages, mesangial cells, endothelial cells, epithelial cells (tubular and glomerular), smooth muscle cells and mitogen stimulated fibroblasts, and have chemotactic and mitogenic effects as well as influence on the accumulation of extracellular matrix. However, as demonstrated by transfection and infusion studies, the overexpression of the different growth factors results in the predominance of different pathological features. Thus, the most prominent consequence of transfection of TGFβ in the kidney is increased accumulation of extracellular matrix (ECM) and glomerular sclerosis, while PDGF induces marked mesangial proliferation [11, 12]. Intravenous infusion of bFGF, in contrast, results in enlargement, vacuolation, and karyomegaly of podocytes in glomeruli, dilation and cast formation in tubules, as well as thickening of the media in the lobular arteries. [13]. Basic FGF is also associated with mesangial cell proliferation in rats pretreated with subnephritogenic doses of anti-Thy-1 antibody [7, 14]. This suggests that variations in the rate of progression of damage in different forms of progressive GN may be linked to an altered fibrogenic growth factor profile. This study investigates the expression of fibrogenic growth factors in patients with IgAN and FSGS, and focuses on the relationship between growth factor expression, interstitial inflammatory cell infiltrate, histopathological changes and the overall severity of chronic renal injury.

Methods

Patients and controls

Studies were performed on renal biopsy tissue from 27 patients with IgAN and 14 patients with FSGS. All biopsies were performed for diagnostic purposes and only a small segment was used for immunohistochemistry in cases in which there was enough material. Patient's clinical details are summarized in Table 1.

Control renal tissue was obtained from the pole opposite to the tumor following a nephrectomy for renal carcinoma. Only those tissues with normal morphology were included as controls in this study.

These studies have been approved by the Human Ethics Review Committee of the Alfred Group of Hospitals.

Histopathology

Renal biopsies were fixed in 10% buffered formalin and embedded in paraffin for routine histological assessment and frozen

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Table 1. Patients's clinical details

	IgAN	FSGS	Controls
Number of patients	27	14	8
Median age (*range*)	36 (19–80)	54 (21–74)	56 (42–63)
Reduced creatinine clearance *< 90 ml/min*	48%[a]	79%	
Proteinuria *> 150 mg/24 hrs*	87%	100%	
Nephrotic *>3 g/24 hrs*	23%	42%	
Haematuria *> 10 RBC/mm³*	96%	38%	
Hypertension	57%	83%	

[a] Percentage of patients

in liquid nitrogen for immunofluorescence or immunohistochemical analysis of IgA, IgG, IgM and C_3, C_4, C_1q and fibrin. Sections for histopathology were stained with Hematoxylin and Eosin, Masson trichrome, Silver Masson trichrome and Periodic Acid-Schiff. All biopsies were also examined by electron microscopy. The diagnosis of IgAN was established by immunohistochemistry showing staining of the mesangium.

The results of histopathological examination of the biopsies are expressed as percentage of IgAN and FSGS patients demonstrating varying degrees of severity of histopathological damage in the glomeruli and the tubulointerstitium.

Immunohistochemical analysis of infiltrating leukocytes, fibrogenic growth factors and their receptors

A small segment of the renal biopsy was embedded in OCT medium (Tissue Tek, Bayer Diagnostics) and frozen in liquid nitrogen. Cryostat sections (4 μ) were cut onto gelatinized slides and post-fixed with acetone (10 min at 4°C), paraformaldehyde-lysine-periodate (PLP) or 4% paraformaldehyde fixative [15]. Acetone and 4% paraformaldehyde fixative were found to best preserve antigenicity, but morphology was better with the latter. PLP fixation was adequate for cell surface antigens, PDGF receptors and bFGF.

The antibodies used in this study are listed in Table 2. A three or four layer immunoperoxidase technique was used for polyclonal or monoclonal antibodies, respectively [18].

Four layer technique. Briefly, nonspecific staining was blocked by preincubation with 10% normal rabbit serum (NBS), 10% fetal calf serum (FCS) in PBS with 0.01% sodium azide (Az) for 10 minutes at room temperature (RT). This was followed by overnight incubation with the primary mouse anti-human antibody at 4°C. After each incubation, slides were washed in phosphate buffered saline (PBS)-0.2% gelatin. A second layer, goat anti-mouse IgG (Sigma) was then applied for 30 minutes at RT. Endogenous peroxidase activity was blocked by incubation for 10 minutes in methanol/0.03% H_2O_2, after dehydration through graded alcohols. After rehydration and washing, slides were incubated with the third layer, rabbit anti-goat immunoglobulin (Dako), followed by the fourth layer, goat peroxidase anti-peroxidase (Dako), both for 30 minutes at RT. The reaction was developed by the addition of Metal Enhanced Diaminobenzidine substrate (Pierce), and slides were counterstained in Harris Hematoxylin, dehydrated, cleared and mounted.

Three layer technique. Sections were preincubated in 10% normal swine serum (NSS) in PBS/Az. Primary rabbit anti-human antibodies were incubated overnight at 4°C. Endogenous peroxidase was blocked as described for the 4 layer technique. The second and third layers were swine anti-rabbit (Dako) and rabbit peroxidase-anti-peroxidase (Dako), respectively, both incubated for 30 minutes at room temperature. Washing steps between incubations, the development of the reaction, counterstaining and mounting were performed as described above.

Negative controls included sections where the primary antibody was omitted for every biopsy. Isotype-specific control monoclonal antibodies (Dako), or a purified, solid-phase absorbed rabbit immunoglobulin fraction from healthy non-immunized rabbits (Dako) were also used. No staining was observed with these irrelevant antibodies.

Antibody absorption

The specificity of the antibodies to PDGF-A and bFGF was confirmed by solid phase absorption studies [19]. Microtiter ELIZA wells were coated with recombinant human PDGF-A (Genzyme) at a concentration of 50 μg/ml in 50 mM carbonate buffer, pH 9.6, bFGF (Bachem Inc.) at 0.1 μg/ml, or carbonate buffer and incubated overnight at 4°C. The antigen solution was then removed and nonspecific binding was blocked by incubation with 1% bovine serum albumin (BSA), 0.02% Az in PBS for two hours at room temperature. Wells were then washed in three changes of PBS and allowed to air dry. Rabbit anti-PDGF-AA (Genzyme) diluted to a final concentration of 10 or 2.5 μg/ml, mouse anti-bFGF (UBI) at 0.5 μg/ml or diluent (1% BSA in PBS) were then added to the wells and incubated overnight at 4°C. The supernatants were then harvested and placed on control kidney sections and staining was carried out as described above. The lower PDGF-AA antibody concentration (2.5 μg/ml) was chosen as the lowest that retained a strong staining pattern on control sections after titering out. Wells coated with PDGF-A and bFGF acted as specificity controls for absorption with anti-bFGF and anti-PDGF-AA antibodies, respectively. Wells coated with carbonate buffer acted as positive controls.

Immunohistochemical assessment of biopsies

The numbers of $CD45^+$, $CD3^+$ and $CD14^+$ leukocytes in the interstitium and in all glomeruli were counted using an ocular grid at a magnification of ×400. Interstitial infiltrate is expressed as number of positive cells/mm^2. Glomerular infiltrate is expressed as number of positive cells/glomerulus.

The expression of growth factors and growth factor receptors, was examined in biopsies and controls. The expression of a particular antigen was defined as being up-regulated when it was increased when compared to controls or when biopsies demonstrated a pattern of distribution different to that of controls.

Image analysis

The extent of expression of glomerular and interstitial PDGF receptor (PDGFR)-β and glomerular PDGF-A was evaluated using a Video Pro computer assisted image analysis system (Leica), with a Leitz Laborlux S microscope and a Panasonic digital camera. Results for interstitial staining represent the area of positive staining in the interstitium expressed as a percentage of the total interstitial area. Positive staining in the glomerulus was expressed as a percentage of the area of each glomerulus. All glomeruli were assessed in each biopsy, and results obtained for individual glomeruli were averaged for each patient. The expression of the other growth factors and receptors was not analyzed in this manner, since the relative lack of definition in the staining

Table 2. Antibodies

Specificity	Antibody	Concentration	Type	Source
CD45 (common leukocyte)	71.5	Supernatant	Monoclonal	Dept. of Medicine [16]
CD14 (monocytes/macrophages)	90.3.8	Supernatant	Monoclonal	Dept. of Medicine [17]
CD3 (T cells)	UCHT-1	3.3 μg/ml	Monoclonal	Dako
bFGF	05-118	0.5 μg/ml	Monoclonal	Upstate Biotechnology Incorporated (UBI)
$TGF\beta_{123}$	2D7/44	30 μg/ml	Monoclonal	Biogenesis
PDGF-AA	ZP-214	10 μg/ml	Polyclonal	Genzyme
PDGF-AA	06-130	10 μg/ml	Polyclonal	UBI
PDGF-BB	ZP-215	20 μg/ml	Polyclonal	Genzyme
PDGFR-β	Code 1263-00	2 μg/ml	Monoclonal	Genzyme
PDGFR-α	Code 1264-00	66 μg/ml	Monoclonal	Genzyme

Table 3. Histopathological assessment of renal biopsies

Histopathology	IgAN	FSGS
	% of patients	
Mesangial proliferation[a]		
Mild	73	57
Moderate	19	0
Severe	4	0
Necrotic lesions	19	0
Crescents[a]	44	0
Glomerulosclerosis		
Global	67[b]	86
Segmental[a]	20	100
Interstitial fibrosis		
Mild	42	36
Moderate	27	29
Severe	12	36
Vascular changes		
Mild	62	14
Moderate	15	36
Severe	4	29

[a] $P < 0.01$ when comparing IgAN and FSGS

[b] Percentage of patients with one or more glomerulus demonstrating global or segmental glomerulosclerosis

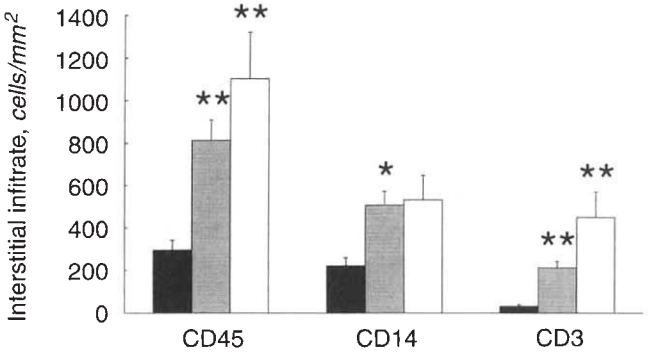

Fig. 1. *Interstitial inflammatory cell infiltrate.* Individual cells in the interstitium were counted using an ocular grid at a magnification of ×400. Results are expressed as number of positive cells/mm². Symbols are: (■) control; (▨) IgAN; (□) FGS; $*P < 0.05$; $**P < 0.01$.

pattern introduced an unacceptable level of error in the detection of positive versus negative signals.

Statistics

Statistical analysis was performed using SPSS for Windows. Comparisons of continuous variables were performed by Kruskall Wallis ANOVA by ranks, with pairwise comparisons analyzed by Mann Whitney *U*-test. Proportions of patients in the different groups were compared by Pearson's χ^2 test, with continuity correction for small sample number. Fisher's two-tailed test was used when the conditions for χ^2 statistics were not met (more than 80% of cells with expected values < 5). Spearman's correlation was performed for association studies.

Results

Histopathological features of patient biopsies

The biopsies demonstrated marked variability in severity of injury (Table 3). All but one IgAN patients demonstrated mesangial proliferation, ranging from mild to severe. This was significantly greater ($P < 0.01$) in IgAN compared to FSGS, in which only mild mesangial hypercellularity was seen in 57% of patients. All but one patient, who had IgAN, demonstrated expansion of mesangial matrix in non sclerosed glomeruli. Global and segmental sclerosis were extensive in FSGS patients, but some degree of glomerular sclerosis was also seen in 67% of IgAN patients, with up to 70% of glomeruli involved. Occasional necrotic lesions and cellular or fibrocellular crescents were observed, but only in IgAN patients. Interstitial injury ranged from mild interstitial inflammatory cell infiltrate with preserved tubules to moderate or severe tubular atrophy, interstitial fibrosis and infiltrate. Most patients demonstrated some degree of vascular damage. Vascular changes ranged from mild hyaline arteriolar sclerosis and mild arteriosclerosis with minimal intimal thickening to severe arteriosclerosis with marked intimal fibrosis and reduplication of internal elastic lamina. As shown on Table 3, there was a tendency for FSGS patients to display more severe vascular and interstitial changes than IgAN patients.

Inflammatory cell infiltrate

The interstitial inflammatory cell infiltrate ranged from very mild to severe in both IgAN and FSGS patients (Fig. 1) with an overall significant increase in $CD45^+$ leukocytes in both forms of glomerulonephritis when compared to control kidneys ($P < 0.01$). The infiltrate was mainly composed of $CD14^+$ monocytes/macrophages and $CD3^+$ T cells, though eosinophils were also occasionally evident. Although some patients demonstrated very marked increases in $CD14^+$ cells when compared to controls there was a wide variability between patients, and no significant differences were detected by Kruskal Wallis ANOVA by ranks. However, a

Table 4. Glomerular changes

	IgAN	FSGS	Control
CD45	10.2 ± 4.7[a]	5.9 ± 1.2	6.9 ± 1.3
CD14	4.2 ± 1.6	2 ± 0.5	1.1 ± 0.3
CD3	0.8 ± 0.4	0.6 ± 0.2	0.8 ± 0.2
PDGF-A	16.7 ± 2.4%[b,c]	9.7 ± 2.3%	5.1 ± 1.3%
PDGF receptor β	22.6 ± 2.2%	18.3 ± 3.5%	14.6 ± 1.7%

[a] Cells per glomerulus (mean ± SEM)
[b] Percentage positive area as assessed by image analysis
[c] $P < 0.001$ when compared to controls

significant difference in $CD14^+$ cell numbers ($P < 0.05$) was detected for IgAN patients versus controls when pairwise comparisons were performed using the Mann Whitney *U*-test. The numbers of interstitial T cells were significantly increased in both IgAN and FSGS when compared to controls ($P < 0.01$).

CD3+ infiltrating cells tended to have a focal distribution in the kidney. In contrast, $CD14^+$ monocytes/macrophages had a diffuse distribution throughout the kidney, particularly localized to areas of damage in which expression of PDGF receptors was demonstrated in expanded intertubular areas of the interstitium.

The glomerular inflammatory cell infiltrate is described in Table 4. No significant increases were demonstrated in either form of GN when compared to controls.

Fibrogenic growth factors and their receptors

Control human kidneys. Figure 2 depicts the patterns of expression of growth factors and receptors in control human kidney. All growth factors were detected on vessels, with bFGF being prominent on endothelial cells (Fig. 2a), and PDGF and TGFβ being expressed by vascular smooth muscle cells. bFGF was also seen on discrete glomerular cells, including mesangial and parietal epithelial cells of Bowman's capsule (Fig. 2b), and on occasional tubular cells, with its characteristic localization in the nucleus, as well as on interstitial cells in the medulla. PDGFR-α was not detected in controls. In contrast, PDGFR-β was strongly expressed by glomerular mesangial cells, occasional parietal epithelial cells and peritubular interstitial cells (Figs. 2 c, d). PDGF-A was striking in its distribution along the tubular basement membrane (TBM) (Fig. 2e). Glomerular staining with anti-PDGF-AA antibodies was either negative or minimal. Equivalent patterns of expression were obtained in 4% paraformaldehyde or acetone fixed tissues. This pattern of PDGF-A expression differs from that described by Alpers et al [19], possibly due to the harsher conditions of tissue processing (paraffin-embedded tissue fixed in methyl Carnoy's fixative) used in that study. Since our initial studies were performed using a Genzyme anti-PDGF-AA antibody, we repeated our staining using one of the antibodies used by their group, the UBI rabbit anti-PDGF-AA. No differences were observed in the staining patterns of these two antibodies, thus confirming our observation of PDGF-A expression along the TBM.

Antigen-specific solid phase absorption of anti-bFGF (0.5 μg/ml) and anti-PDGF-AA antibody at 2.5 μg/ml removed the staining seen on renal sections. Absorption of the PDGF-AA antibody at 10 μg/ml resulted in diminished staining, but the antigen coating the ELIZA wells was not sufficient to completely abolish reactivity with the tissue section. Incubation in wells containing either coating buffer only or an irrelevant antigen did not affect the pattern or intensity of either antibody, thus confirming the specificity of the staining.

Up-regulated patterns of expression of growth factors and growth factor receptors

Despite variations in the degree of up-regulation of growth factors and receptors between individuals, specific staining patterns were observed for each protein. Figure 3 depicts representative examples of altered expression of growth factors and receptors in the glomeruli and in the interstitium.

In the glomerulus, the most striking changes were seen in the expression of PDGF-A and PDGF receptors. PDGF-A was seen along the capillary loops, as well as in infiltrating leukocytes and some intrinsic glomerular cells, possibly mesangial (Fig. 3 a, b). PDGF receptors were prominent in areas of mesangial expansion (Fig. 3 c, e). PDGF-B and bFGF (Fig. 3g) were occasionally seen segmentally in glomeruli, and TGFβ was occasionally expressed by few cells in the glomeruli (Fig. 3i).

In the interstitium, PDGF-A (Fig. 3b) and PDGF-B were up-regulated in collapsed, atrophied tubules and in vascular structures. The expression of PDGFR-α (Fig. 3d) and in particular of PDGFR-β (Fig. 3f) was markedly increased in expanded intertubular areas of fibrosis. Basic FGF was increased in areas of tubular atrophy and interstitial fibrosis, and appeared to be expressed by tubular epithelial cells as well as by fibroblasts and myofibroblasts and occasionally infiltrating leukocytes in these areas (Fig. 3h). TGFβ expression tended to be very restricted, with either few infiltrating cells, occasional tubular cells or few vascular structures showing any significant changes (Fig. 3j).

Expression of growth factors and growth factor receptors in IgAN and FSGS

Both patient groups demonstrated marked variability in expression of growth factors and receptors, and the degree of up-regulation seen in each biopsy appeared to be associated with the severity of injury, and with the extent of mononuclear cell infiltrate.

Changes in expression of glomerular PDGF-A and glomerular and interstitial PDGFR-β in patients and controls were quantified by image analysis. As shown on Table 4, glomerular PDGF-A was significantly increased in patients with IgAN when compared to controls ($P < 0.001$), while no significant difference was evident in the FSGS group. In contrast, no significant differences were detected between the groups when image analysis of glomerular PDGFR-β was performed, while interstitial PDGFR-β was significantly increased in both IgAN and FSGS patients when compared to controls ($P < 0.01$ and $P < 0.05$, respectively; Fig. 4).

Further comparisons between IgAN and FSGS patients were based on the relative proportions of patients with either form of GN demonstrating changes in expression of growth factors and receptors (Table 5). The percentages of FSGS patients with abnormal expression of growth factors and receptors in the interstitium was higher than in IgAN, and this attained significance for bFGF ($P < 0.005$), while a clear trend was evident for PDGF-A and PDGF-B. No significant differences were seen in the proportions of patients with altered expression of growth factors in the glomerulus.

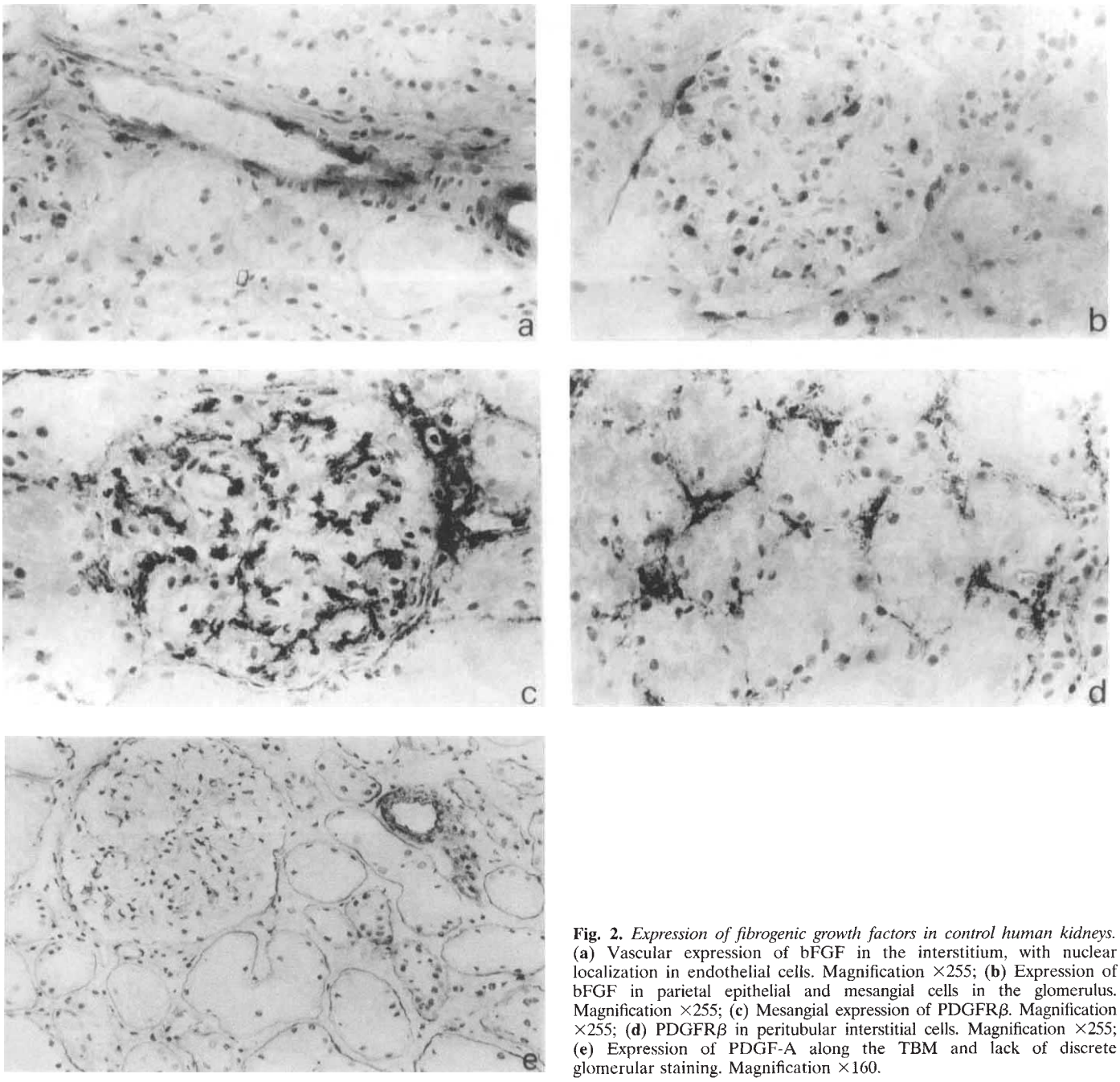

Fig. 2. *Expression of fibrogenic growth factors in control human kidneys.* (**a**) Vascular expression of bFGF in the interstitium, with nuclear localization in endothelial cells. Magnification ×255; (**b**) Expression of bFGF in parietal epithelial and mesangial cells in the glomerulus. Magnification ×255; (**c**) Mesangial expression of PDGFRβ. Magnification ×255; (**d**) PDGFRβ in peritubular interstitial cells. Magnification ×255; (**e**) Expression of PDGF-A along the TBM and lack of discrete glomerular staining. Magnification ×160.

Correlation between interstitial PDGFR-β expression and CD14$^+$ infiltrate

As already stated, the CD14$^+$ monocytes/macrophages had a diffuse distribution throughout the kidney, and were particularly localized to areas of damage with increased expression of PDGF receptors in the interstitium. To investigate this further, the expression of PDGFR-β was correlated with CD14$^+$ infiltrating cells in the interstitium. A highly significant positive correlation was demonstrated, as shown in Figure 5, with a Spearman correlation coefficient of 0.65 ($P < 0.0001$).

Discussion

The involvement of fibrogenic growth factors in chronic renal injury has been demonstrated in a variety of clinical and experimental studies. This study has focused on the expression of fibrogenic growth factors and their relation to severity of injury in two contrasting types of GN, IgAN and FSGS. The expression of growth factors in GN patients was also compared with that seen in control human kidneys.

PDGF is a potent mitogen and chemoattractant for mesenchymal cells, mesangial cells and monocytes/macrophages. It causes

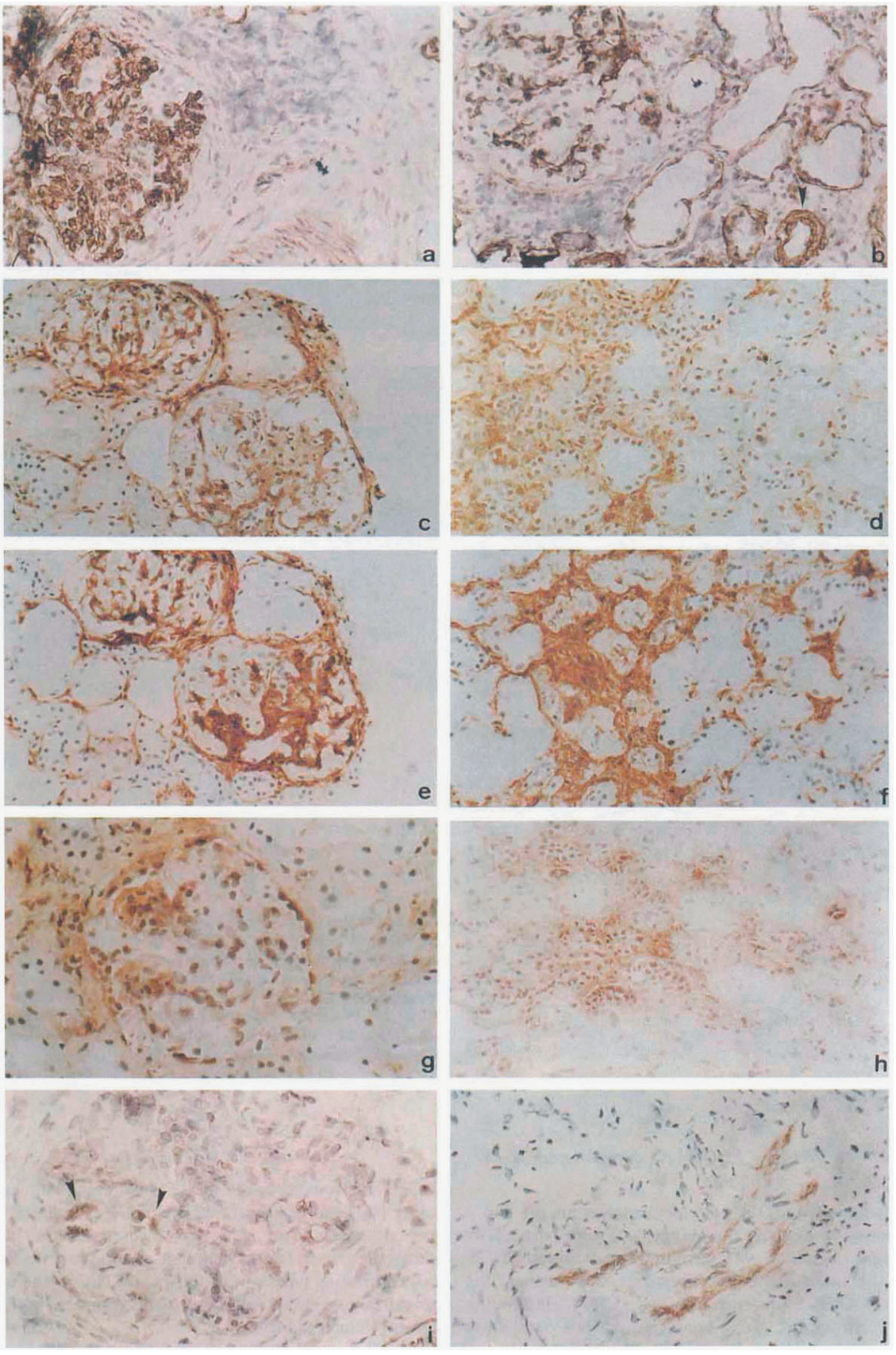
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Fig. 3. *Altered expression of fibrogenic growth factors in patients with GN.* (**a**) PDGF-A staining along the capillary loops and in the mesangium of a glomerulus from a patient with IgAN. Magnification ×140; (**b**) Up-regulation of PDGF-A expression in the glomerulus and in atrophied tubules (arrowhead) in an IgAN patient. Preserved expression along the TBM. Magnification ×140; (**c**) Up-regulated glomerular expression of PDGFRα in expanded mesangial areas and staining of peritubular interstitial cells in a patient with IgAN. Magnification ×140; (**d**) Expression of PDGFRα in expanded intertubular areas in an FSGS patient. Magnification ×140; (**e**) Same patient as in "c" demonstrating upregulation of PDGFRβ. Magnification ×140; (**f**) Same patient as in "d", with up-regulated expression of interstitial PDGFRβ. Magnification ×140; (**g**) Glomerular expression of bFGF in a patient with IgAN: nuclear expression of bFGF is evident in parietal epithelial cells, while extracellular bFGF is seen segmentally in the mesangium. Staining of interstitial cells is also evident. Magnification ×220; (**h**) Interstitial bFGF demonstrated on tubular epithelial cells and interstitial cells in an FSGS patient. Magnification ×140; (**i**) Up-regulated expression of TGFβ in discrete cells within the glomerulus (arrowheads) in an IgAN patient. Magnification ×220; (**j**) Prominent expression of TGFβ in a partially occluded artery from an IgAN patient. Magnification ×140.

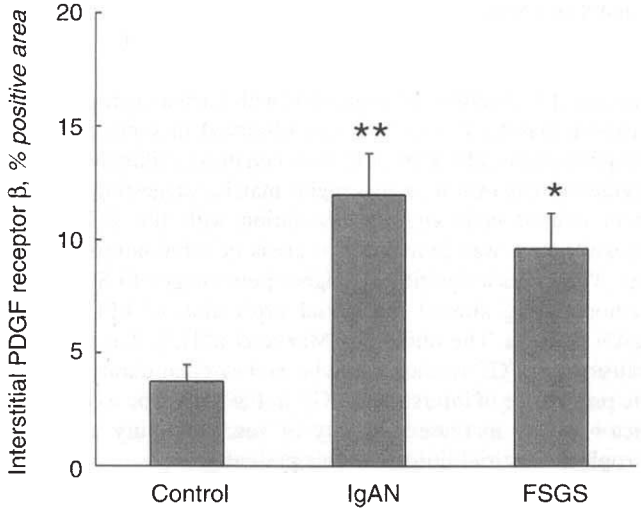

Fig. 4. *Interstitial expression of PDGFRβ.* The area of positive staining for PDGFRβ in the interstitium was measured by computer assisted image analysis, as a percentage of the total area of interstitium. Interstitial PDGFRβ was significantly increased in IgAN patients ($^{**}P < 0.01$), and in FSGS patients ($^{*}P < 0.05$) when compared to controls.

cell contraction and stimulates release of other cytokines such as TGF-β [9, 20, 21]. Two subunit chains of PDGF, A and B, combine to form the dimers PDGF AA, BB or AB. PDGFR-α binds both A and B PDGF chains, and mediates mitogenesis, while PDGFR-β only binds the PDGF-B chain, and mediates mitogenesis, chemotaxis and circular actin reorganization [22].

In the control kidneys, PDGF-A was prominently demonstrated along the TBM, as well as in vascular smooth muscle cells in arteries, also demonstrating PDGF-B. PDGFR-α was not detected, while PDGFR-β was constitutively expressed in glomerular mesangial cells, occasional parietal epithelial cells and cortical and medullary peritubular interstitial cells, as previously described by Alpers et al [23].

The distribution of PDGF-A along the TBM in patients and controls and along the glomerular capillary loops in some patients suggests an association with the ECM. There are two alternative splice isoforms of PDGF-A, differing in the presence or absence of a short exon encoding a highly basic carboxyterminal tail. This results in a shorter isoform, which has been shown to be secreted efficiently from the producer cell, and a longer isoform (PDGF-A_L) which remains cell associated, and which has been shown to bind to heparan sulfate proteoglycans in the ECM [22, 24]. Thus, the PDGF-A detected along TBM may represent the PDGF-A_L variant.

Varying proportions of IgAN and FSGS patients demonstrated an up-regulation of PDGF ligand and receptors, depending on the extent and severity of renal damage. Marked changes in expression of PDGF-A were seen in the glomerulus, where it was detected in the mesangium and on capillary loops in most patients. The degree of up-regulation was variable, however, and image analysis demonstrated a significant increase in PDGF-A expression in IgAN but not in FSGS with respect to controls. The strong mitogenic effect of PDGF on mesangial cells suggests the prominence of this growth factor in IgAN may be linked to the mesangial proliferation observed in this patient group.

The increases in glomerular expression of PDGF ligand and receptor β observed in this study are in agreement with previous findings in experimental and clinical glomerulonephritis [3, 8, 25–29]. However, the alterations observed in this study in receptor α expression have not been previously reported in IgAN or FSGS. In fact, Gesualdo et al [25] were not able to demonstrate this in a study of biopsies of patients with various forms of GN, other than in few patients with lupus nephritis. In the same study, they also report constitutive low level expression of PDGFR-α in glomeruli and interstitium of controls, which we did not observe. The presence of very few or no α receptors in conjunction with the constitutive expression of PDGF-A may represent a mechanism to prevent the autocrine action of PDGF-A in normal renal tissue, as suggested by Abboud [30]. In this context, the up-regulation of PDGFR-α that we demonstrate in disease would represent the failure of such mechanism, ultimately contributing to the chronic renal injury.

The up-regulation of PDGF receptors, and particularly of PDGFR-β in the interstitium was one of the most striking changes observed in these biopsies. We have observed that in biopsies with features of tubulointerstitial damage, up-regulation of PDGFR-β occurred concomitantly with increased expression of α-smooth muscle actin, suggesting that the receptor was mainly expressed on myofibroblasts (data not shown). The increased expression of PDGF receptors was consistently accompanied by infiltrating monocytes/macrophages, resulting in a highly significant correlation between these pathological features. In contrast, the distribution of T cells was mostly focal and was not spatially associated with growth factor or receptor expression. Macrophages have been implicated in the pathogenesis of various models of renal disease [reviewed in 31] and are known to produce a number of cytokines which lead to the activation and proliferation of glomerular, tubular and interstitial cells, including myofibroblasts. These cytokines include TNF-α, IL-1β, bFGF, TGFβ and PDGF among others, and their release may be an important factor in upregulating the expression of PDGFR-β as detected in this study. Conversely, fibrogenic growth factors and other cytokines such as IL-8, macrophage chemotactic peptide (MCP-1) and the glycoprotein osteopontin released by tubular epithelial cells, other

Table 5. Expression of growth factors and their receptors in IgAN and FSGS

Growth factor/receptor	Interstitium		Glomeruli	
	IgAN	FSGS	IgAN	FSGS
TGFβ_1	30%[a]	54%	11%	0%
bFGF	33%	86%[b]	28%	30%
PDGF-A	40%	75%[c]	72%	60%
PDGF-B	32%	67%[c]	6%	22%
PDGF receptor α	48%	50%	33%	10%
PDGF receptor β	56%	79%	58%	50%

[a] Percentage of patients with upregulated expression of growth factor/receptor
[b] $P < 0.005$ Pearsons χ^2 test; $P < 0.005$, continuity correction when comparing IgAN vs. FSGS
[c] $P < 0.05$ Pearsons χ^2 test; $P = 0.1$, continuity correction when comparing IgAN vs. FSGS

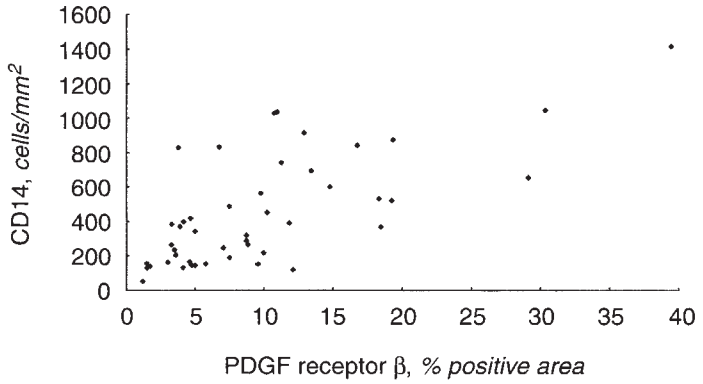

Fig. 5. *Correlation between interstitial PDGFRβ and monocyte/macrophage infiltrate.* The percentage positive area of PDGFRβ expression in the interstitium was assessed by image analysis. The numbers of $CD14^+$ cells (monocytes/macrophages) were counted using an ocular grid at a magnification of ×400, and are expressed as cell/mm^2. $P < 0.0001$. Spearman's correlation was performed using SPSS for Windows.

interstitial cells and by activated myofibroblasts are chemotactic for the macrophages, and this, together with the up-regulation of adhesion molecules contributes to their localization in areas of injury, thus perpetuating and amplifying the activating and fibrogenic signals in the interstitium.

Basic FGF is a powerful mitogen and chemotactic factor for mesangial cells, smooth muscle cells, fibroblasts and macrophages [32, 33], and it has been implicated in various experimental models of renal disease, particularly in the initiation phase of mesangial proliferation [7, 8, 10, 34]. There is very little information regarding the localization of bFGF in normal kidney, and the pattern observed in our control kidneys was consistent with our findings in the rat. There was constitutive expression of bFGF in vascular endothelial cells, on discrete mesangial and parietal epithelial cells of Bowman's capsule and on interstitial cells in the medulla, and a nuclear localization of bFGF was frequently observed. This compartimentalization can be explained by the findings of Patry et al [35], who demonstrated that internalized bFGF in endothelial cells can then be located either in the cytoplasm or the nucleus, the nuclear translocation occurring in the late G_1 and S phases of the cell cycle. It is of interest that although the translocation pathway and its biological significance is not clear, it appears that the presence of FGF in the nucleus can be correlated with the mitogenic response.

In GN patients, the normal pattern of expression of bFGF was preserved, but additional expression with nuclear, cytoplasmic and extracytoplasmic localization was observed in some patients. In the glomerulus, bFGF protein was seen most strikingly in areas of segmental expansion of mesangial matrix, suggesting its release from injured cells and its association with the ECM. In the interstitium it was prominent in areas of tubulointerstitial damage. There was a significantly higher percentage of FSGS patients demonstrating altered interstitial expression of bFGF than of IgAN patients. The finding by Mazue et al [13], that intravenous infusion of bFGF results in tubular and vascular damage, suggests the prevalence of interstitial bFGF in FSGS may be a contributing factor to the increased severity of vascular injury and tubular atrophy/interstitial fibrosis in this patient group.

TGFβ is a multifunctional growth factor, synthesized by many cell types including renal tubular epithelial cells, mesangial cells and macrophages [9, 36, 37]. It has important effects resulting in the accumulation of extracellular matrix, and is also a strong chemoattractant for monocytes/macrophages and fibroblasts. TGFβ has been implicated in the development of injury in GN [8, 28, 38–42]. We have also detected increased expression of TGFβ mRNA in chronic renal allograft rejection [43] and in the 7/8 nephrectomy model of FSGS [44]. The limited expression of TGFβ detected in biopsies of patients with GN was therefore unexpected, and may reflect the specificity of the monoclonal antibody used in this study, which may not recognize all active or latent forms of TGFβ. In fact, different staining patterns obtained with antibodies to different epitopes of TGFβ_1 have been described previously [45].

In conclusion, this study has demonstrated elevated expression of PDGF, PDGF receptors and bFGF in patients with IgAN and FSGS. Specific patterns of up-regulation were evident, strongly associated with the severity and type of injury. The active involvement of monocytes/macrophages in the development of chronic injury was confirmed by a strong correlation between their numbers and localization in the interstitium and the phenotypic change of fibroblasts to myofibroblasts, with strong expression of PDGF receptors. The expression of PDGF-A in the glomerulus was up-regulated in IgAN as compared to FSGS patients, suggesting an association with mesangial proliferation. Conversely, FSGS patients demonstrated higher expression of PDGF-A and -B as well as bFGF in the interstitium, reflecting the relatively more severe degree of vascular and tubulointerstitial injury seen in this patient group. In contrasting these two different types of GN, this study was thus able to identify links between overexpression of particular growth factors in the various compartments of

the kidney with associated injury, and in this way contributes to the definition of the relevant pathways of damage in renal disease.

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